Dot Blot

A technique for detecting, analyzing, and identifying proteins, similar to the western blot technique but differing in that protein samples are not separated electrophoretically but are spotted through circular templates directly onto the membrane or paper substrate. Concentration of proteins in crude preparations (such as culture supernatant) can be estimated semi-quantitatively by using “Dot Blot” method if you have both purified protein and specific antibody against it.

This protocol comes from *DNA Methylation on N6-Adenine in C. elegans, Shi et al., Cell, 2015*.

Materials :

- Extracted worm DNA
- Hybond
- Membranes
- Primary antibody α 5mC or 6mA
- Secondary antibody

Reagents:

- TBS (20 mM Tris-HCl 150 mM NaCl pH 7.5)
- TBS-T (0.05% Tween20 in TBS)
- BSA/TBS-T (0.1% BSA in TBS-T)
- Nitrocellulose membrane

Procedure:

- Samples were diluted to 100 ng/ml and heated at 95C for 10 min to denature DNA.
- Samples were immediately placed on ice for 5 min, and 250 ng were loaded per dot on Hybond + membranes.
- Membranes were allowed to air dry and placed in boxes with damp paper towels.
- DNA was then autocrosslinked in a UV stratalinker 2400 (Stratagene) two times.
- The membrane was allowed to dry and then blocked for 1 hr in 5% milk TBS.
- Membranes were probed for 1 hr at room temperature or overnight at 4C with primary antibody in 5% milk TBS.
- Blots were washed three times for 10 min with TTBS and then probed with secondary antibody in 5% milk for 1 hr at room temperature.
- Blots were washed three times for 10 min with TTBS, and ECL was applied and film was developed.